SPECIAL REPORT 95-20

CRREL

Development of a Field Method for Quantifying Ammonium Picrate and Picric Acid in Soil and Water

Philip G. Thorne and Thomas F. Jenkins

August 1995

Abstract

Methods for the detection and quantification of ammonium picrate and picric acid in soil and water were developed. Picrate ions were extracted from water directly or from acetone extracts of soil by solid-phase, acidic, ion-exchange materials. Elution from the ion exchangers was accomplished by converting the retained picrate to picric acid using strong aqueous, acid–organic solvent mixtures. The resulting colorless solution was then converted back to a colored picrate solution by dilution with water. Quantification and correction for background interferences were based on spectrophotometric measurements. A colorimetric, chemical confirmation of picrate was possible for the water method. The method detection limits were determined to be 1.3 µg/g for soil and 3.6 µg/L for water. Both methods can be implemented under field conditions.

For conversion of SI units to non-SI units of measurement consult ASTM Standard E380-93, *Standard Practice for Use of the International System of Units,* published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.

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US Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

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Prepared for
U.S. ARMY ENVIRONMENTAL CENTER
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PREFACE

This report was prepared by Philip G. Thorne, Research Physical Scientist, and Thomas F. Jenkins, Research Chemist, Geological Sciences Division, Research and Engineering Directorate, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL), Hanover, New Hampshire. Funding was provided by the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland, Martin H. Stutz, project monitor.

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Development of a Field Method for Quantifying Ammonium Picrate and Picric Acid in Soil and Water

PHILIP G. THORNE AND THOMAS F. JENKINS

INTRODUCTION

Source, fate and toxicology of ammonium picrate and picric acid

Ammonium picrate (ammonium 2,4,6-trinitrophenoxide, CAS 131-74-8)—Explosive D—was used in armor-piercing shells, bombs and rocket warheads by the U.S. military from the turn of the century to after World War II. It is no longer manufactured but now represents 8% of the demilitarization inventory. Picric acid (2,4,6-trinitrophenol, CAS 88-89-1) was used as a grenade and mine filling (Meyer 1987) (Fig. 1).

Layton et al. (1987) reported comprehensively on the production, toxicology and environmental fate of ammonium picrate and its parent compound, picric acid. When dissolved in water, both ammonium picrate and picric acid dissociate to the picrate ion. Aqueous solubilities for both compounds are over 10 g/L, and they appear to present an extremely mobile environmental contaminant. Goodfellow et al. (1983) showed that the partitioning of picrate from estuarine water to organic sediment was very low. This follows from the low octanol–water partition coefficient for picric acid (log K_{ow} = 1.6) (Layton et al. 1987).

On the other hand, Layton et al. (1987) predicted that picrate will act like phenolic pesticides and become incorporated into or bound to humic substances. Chang and Anderson (1968) studied the flocculation of clays by picric acid and found that the degree of flocculation depended on the nature of the clay and associated ions. When picric acid was mixed with solutions containing calcium ion and calcium clays, flocculation occurred rapidly and completely, removing picrate from solution. Mixtures containing sodium ions and sodium clays formed stable suspensions, with picrate remaining in solution. Mixtures of sodium ions with calcium clays or calcium ions with sodium clays produced intermediate effects. These experimental studies suggest that transport of picrate in the environment will be highly variable, depending on the organic and mineral composition of each soil. Kayser and Burlinson (1988) found that picrate migrated through four soils in lysimeter studies. Van Denburgh (reported in Layton et al. 1987) found that picrate had migrated from a disposal bed at an ammunition plant. Ruchholt and Norris (1946) reported finding one soil that retained "appreciable" amounts of picrate.

Most of the toxicological work reported by Layton et al. (1987) was on skin adsorption and inhalation of ammonium picrate dust. Few data were available on the chronic effects of ingestion of picrate. The most recent research (Wyman et al. 1992) deals with lethal-dose determinations. The EPA has not set an action level for ammonium picrate or picric acid in soil or water. Layton et al. (1987) estimated an Allowable Daily Intake (ADI) of 1–37 µg/kg-d. Since the estimated ADI is similar to other secondary explosives, similar field detection limits were sought in this research (i.e., low µg/g in soil and low µg/L in water). There is potential for picric acid to be transformed to

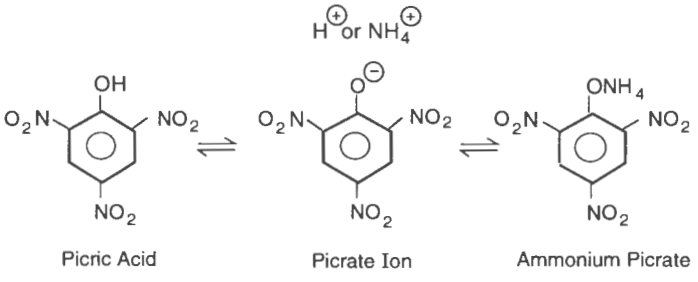

Figure 1. Chemical structures of picric acid, picrate ion and ammonium picrate.

picramic acid (2-amino-4,6-dinitrophenol) by adapted bacteria under the anaerobic conditions that might be found at some waste sites. This compound has ten times the mutagenicity of picric acid (Wyman et al. 1979). The toxic effects on aquatic organisms are also greater for picramic acid than for picric acid (Goodfellow et al. 1983). Picramic acid is also a mammalian metabolic transformation product of picric acid (Barral 1915, Wyman et al. 1992) and is excreted in the urine. It will be introduced into the environment if picric acid is ingested.

Unlike many of the other high explosives that are no longer manufactured and that present environmental clean-up problems unique to the military, picric acid is a ubiquitous industrial chemical. It is widely used as a metal-etching chemical and as feed stock in many processes in the dye, leather and glass industries (Wyman et al. 1992). Picrate is also an environmental transformation product of tetryl, another obsolete military explosive (Kayser et al. 1984). Picrate was detected in a leachate from soil columns spiked with tetryl (Kayser and Burlinson 1988) and was recently detected as a transformation product of tetryl in water (Jenkins et al. 1995). A rapid screening method for the detection of picrate in soil and water will have broad utility. Indications are that it is not degraded in the environment, either biologically or photochemically (Layton et al. 1987), although some strains of adapted organisms may make bioremediation a possibility (Wyman et al. 1979, Lenke and Knackmuss 1992).

Previous analytical methods

Detection of picric acid has been a goal of analytical chemists since the early 20th century, when malingerers ingested picric acid to mimic the symptoms of jaundice to avoid military service. Early detection schemes used colored precipitates (Barral 1915), colored solvent interfaces (Rodillon 1915) or colored solutions (Ydrac 1916) to identify picric acid and its metabolic by-product, picramic acid. In 1923, Deniges investigated the unique color-changing behavior of the picric acid–picrate system in aqueous and strongly acidic, organic solutions.

Forensic analysts have been required to identify and quantify picric acid in complex mixtures of other nitroaromatic explosives. Paper chromatography (Barnabas 1954, Colman 1962) or thin-layer chromatography (Parihar et al. 1966, Bagnato and Grasso 1986) were used to separate picric acid from other explosives, where it was detected by color-forming reagents. Quantification was possible using a photodensitometer (Wyman et al. 1979).

Contemporary analytical methods for the determination of picrate in the environment have focused on extraction, separation from matrix components and analysis in the laboratory. U.S. Geological Survey methods (Goerlitz 1979) used benzene or methylene chloride for extraction from soils and water, followed by concentration, solvent exchange and reverse-phase, high-performance liquid chromatography (RP-HPLC) with eluents containing an ion-pairing reagent. U.S. Army Environmental Center (USAEC) Method LW-13 (1989) used a 10% aqueous methanol solution for extraction from soil, followed by analysis by RP-HPLC. Methylene chloride was used to extract picric acid from acidified water samples, followed by concentration, solvent exchange and analysis by RP-HPLC using a buffered, acidic aqueous–acetonitrile eluent. Midwest Research Institute methods (Conrad 1990a,b) used ion-pairing and solid-phase extraction of picrate ion pairs from water, followed by ion-pair analysis using HPLC with a polycyclic aromatic hydrocarbon column. Picrate was extracted from soils with an acidic methanol–water mixture and analyzed by RP-HPLC using ion-pairing conditions. Lloyd (1985) used RP-HPLC with a buffered, acidic aqueous–methanol eluent to detect picrate and other high explosives from forensic samples. Voyksner and Yinon (1986) used thermospray HPLC-mass spectrometry with chemical ionization to analyze acetone wipes of skin contaminated with six high explosives, including picric acid. Munder et al. (1990) developed a capillary supercritical fluid chromatographic technique that could resolve picric acid from other explosives in complex mixtures.

Ammonium picrate and picric acid are not currently target analytes in SW846 Method 8330 (USEPA 1990). If the standard conditions for extraction and analysis of explosives in soil from Method 8330 are used, picrate would be extracted by overnight sonication in acetonitrile but would not be retained by the RP-HPLC with a 50% aqueous–methanol eluent. Method 8330 for water uses the same separation, and hence, picrate would not be detected using this protocol.

A field screening method for Dinoseb (2-sec-butyl-4,6-dinitrophenol, CAS 88-85-7) in soil was developed by Anderson et al. (1993). Dinoseb is a pesticide that is structurally similar to picric acid but is less acidic and more hydrophobic (Fig. 2). In this method, soil is extracted with methylene chloride and the extract is mixed with Florisil, a basic,

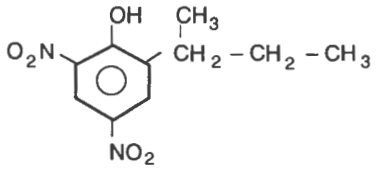

Figure 2. Chemical structure of Dinoseb.

normal-phase extraction material. When Dinoseb is present, the white Florisil turns yellow. A visual detection limit of 5 µg/g was reported; however, quantification of contamination at higher levels was not suggested.

Objectives

The major goal of this effort was to develop a field screening method for ammonium picrate and picric acid that could be used in conjunction with field screening methods already established for the high explosives RDX, TNT and 2,4-DNT (Jenkins and Walsh 1992, Jenkins et al. 1994). If detection limits and action levels are similar, qualitative identification and confirmation of picrate in soil and water is a reasonable stopping point for a screening method. However, if the screening can be semiquantitative or quantitative, then site managers can be alerted to contaminant levels that might produce a significant release if the site is further disturbed. This is particularly true in the case of picrate, where it could conceivably be exposed to water after having been sequestered in clay or sheltered from rain by pavement or buildings. Furthermore, it is hoped that a rapid and inexpensive field test will allow screening for picrate at nonmilitary sites.

The strategy that was employed in this research was to investigate the adaptability of some existing screening methods that detect related compounds and to develop new methods based on the behavior of picric acid as it changes from a colorless, undissociated acid to a yellow picrate ion. The other characteristic of picrate that might allow selective separation from other yellow compounds discussed below is its high acidity. Ion exchange materials and binding and elution conditions were sought that would retain, then release, picrate ions selectively, free from interferences.

EXPERIMENTAL METHODS

Analytes used for spikes were Standard Analytical Reference Material from the U.S. Army Environmental Center. Field-contaminated soils from Hawthorne Army Ammunition Plant (AAP), Hawthorne, Nevada; Naval Surface Warfare Center (NSWC), Crane, Indiana; and Nebraska Ordinance Plant, Mead, Nebraska, were used to test the methods. Well waters from NSWC were also used.

All solvents used for extraction, collection and elution were HPLC-grade from Baker. Reagent-grade water was prepared using a Milli-Q Type 1 Reagent-Grade Water System (Millipore). The solid-phase extraction (SPE) materials were Florisil (Supelco), Alumina-A SPE cartridges (3 mL-1 g, Supelco) and Anion-Empore SPE membranes (47 mm, 3M-Varian). The syringe filters were Millex SR (0.45 µm, 25 mm, Millipore). Immunoassay kits for TNT were EnviroGard (Millipore) and D-TECH (EM Science). The color reagent for nitroaromatics was from the TNT kit from EnSys (Research Triangle Park, North Carolina).

Analyses of picrate and other explosives were performed by RP-HPLC on a 25- × 4.6-cm (5 µm) LC-18 (Supelco) column. The analytes were eluted using 1.5 mL/min of 60:40 (v/v) aqueous buffer:methanol. The buffer was 0.05 M KH_2PO_4, adjusted to pH 3.5 with acetic acid. The analytes were detected at 365 nm for picrate and 254 nm for all others. The spectrophotometer used for the field method was a battery-powered Hach DR-2000 (Loveland, Colorado).

RESULTS

Field screening in soil

Extraction conditions

An initial experiment investigated the potential for adapting the Dinoseb/Florisil method (Anderson et al. 1993) for the detection of ammonium picrate and picric acid. When picric acid was dissolved in $MeCl_2$, it produced a colorless solution characteristic of solvated, but undissociated, picric acid (Majersky and Dybalova 1975). When white Florisil powder was added, the solution remained colorless while the powder turned a brilliant yellow. The color was due to the formation of picrate ion pairs on the basic sorption sites of the Florisil.

The application of this ion-exchange reaction to soil samples was then investigated using 2 g of soil mixed with 5 mL of $MeCL_2$ in a 22-mL glass vial. When wetted soils were extracted, phase separation of the $MeCL_2$ was difficult to achieve if there

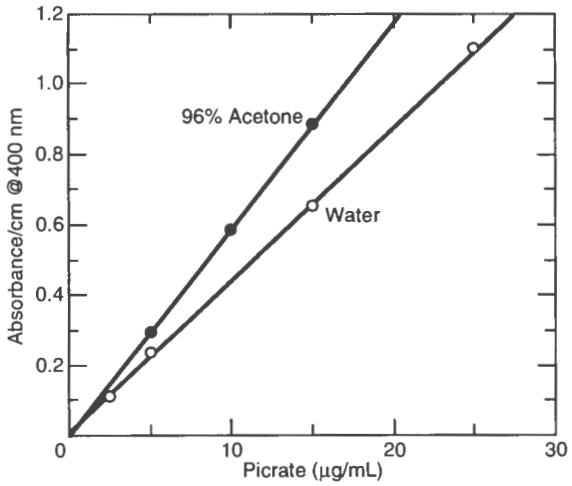

Figure 3. Absorbances for picrate in acetone and water.

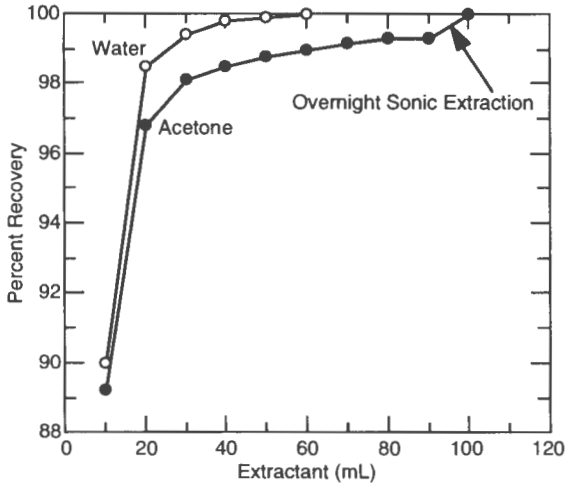

Figure 4. Recoveries of picrate from Hawthorne soils by sequential 10-mL extractions.

was any fresh plant material or humus present. This problem could be overcome with larger volumes of solvent, but this seemed environmentally unwise. Since $MeCl_2$ is denser than water, it collects at the bottom of extraction vessels, dictating the use of separatory funnels. These are expensive and difficult to use and clean in the field. Several other less-toxic solvents with densities less than water were tried to see if they would extract picric acid and exchange it onto Florisil. Isooctane produced a clear extract that, when picric acid was present, turned Florisil yellow, but the extraction efficiency of picric acid from a field-contaminated soil was very low. Ethyl ether was an efficient extractant but resulted in a yellow extract, since it also dissolves some aqueous picrate ions. No exchange with Florisil was apparent. No further experiments were conducted using nonpolar solvents and Florisil.

Several polar solvents (acetone, methanol, isopropanol, acetonitrile and water) were used to extract a soil from Hawthorne AAP known to be contaminated with picric acid. All extracts were yellow. By far the brightest yellows resulted from the acetone and water extractions. This soil had a moisture content of 4%, so standard curves for picrate in 96% acetone/4% water and 100% water were constructed and showed a linear relationship between absorbance at 400 nm and concentration (Fig. 3). The visual detection threshold for picrate in an acetone or water extract corresponds to a soil contamination value of approximately 5 µg/g. The extraction efficiencies of acetone and water were then determined. A 2-g sample of soil was placed in a 22-mL glass vial with 10 mL of extractant and shaken manually for three minutes. The vials were then centrifuged for three minutes and the extractant decanted and filtered through a 0.45-µm Millex SR syringe filter. The quantity of picrate extracted was determined spectrophotometrically. Further aliquots (10 mL) of extractant were added to the soil and the procedure repeated until no more yellow color was extracted. Since the acetone extractions appeared to reach a plateau, the vial containing the tenth aliquot was placed in a sonic bath overnight. This process removed the remaining picrate. Recoveries of picrate from this soil were calculated as a normalized percentage based on the total amount recovered from each sample. The percent recoveries in the first 10-mL aliquot were 89% for acetone and 90% for water (Fig. 4).

Selection of ion exchange materials and binding and elution conditions

Two ion-exchange materials were chosen for investigation. Alumina-A solid-phase extraction cartridges are used in the RDX field method (Jenkins and Walsh 1992). Empore Anion extraction membranes were selected because they have an allowable flow rate approximately 20 times greater than the extraction cartridges and would provide a different exchange chemistry. Solutions containing 1 µg/mL of picric acid in acetone or water were passed over Empore Anion membranes and Alumina-A cartridges. For the acetone solutions, both sorbants became yellow, indicating the retention of picrate ions. The Anion membrane

was more intensely yellow than the Alumina-A since all the picrate was sorbed on the top surface, as opposed to being distributed within the sorbant bed of the cartridge. The Alumina-A did not retain picrate from water only, whereas the Anion membrane showed its greatest retention from water.

Both sorbants that retain picrate are ion-exchange materials; therefore, elution and quantification of the picrate is feasible. For the Anion disk, 10% concentrated sulfuric acid/methanol (v/v), and for the Alumina-A cartridge 2% concentrated sulfuric acid/acetone (v/v), were the mildest conditions that resulted in elution. Lower concentrations of acids, different acids or different solvents were not successful. Under conditions of such high acidity, the picrate is converted to undissociated picric acid and is colorless, as it is in $MeCl_2$. The eluted solution is filtered through a Millex SR syringe filter placed on the tip of the cartridge, then diluted with water until the pH was raised above the pKa of picric acid, resulting in the formation of the colored picrate anion. This enabled both quantification using a field-portable spectrophotometer and confirmation by the unique color-changing behavior of picric acid to picrate (Deniges 1923). The eluent from the Empore Anion membrane did not require additional filtration.

Optimization of conditions

Although the Empore Anion membrane did retain picrate from both acetone and aqueous extracts, it was not chosen for the soil method because one membrane costs nearly ten times as much as one Alumina-A cartridge.

Acetone extracts of field-moist soils will contain variable amounts of water. Since the initial retention experiments showed that the Alumina-A cartridge did not retain picrate from water alone, an additional test was run to determine what percentage of water in acetone would result in the maximum retention of picrate. The results are listed in Table 1. The maximum occurred at a broad plateau around 50% water. The sharp rise in recovery from low water contents to 50% indicated that extracts of field-moist soils would produce unpredictable results if not diluted with water up to the 50% plateau.

Table 1. Recovery of 4.8 µg/mL picrate from water–acetone mixtures.

Water–acetone (%)	*Recovery of picrate (%)*
8	4
25	38
50	86
75	85

The field methods for TNT, 2,4DNT and RDX specify the use of 100 mL of acetone to extract 20 g of soil. These methods use about 60 mL of the common extract for analyses. This leaves 40 mL for the picrate assay. Since it is difficult to remove all of the remaining acetone from the extraction bottle, 30 mL of acetone extract diluted one-to-one with water was chosen for subsequent tests.

Further increases in recovery were realized by optimizing extraction and elution conditions. Breakthrough tests were conducted at different extraction rates using 60 mL of a 50% aqueous–acetone solution fortified with picric acid. At a flow rate of 10 mL/min, about 4% of the picrate was not retained. The loss increased from 4% to 13% when the flow rate was increased to 40 mL/min. Elution with a 10-mL aliquot of 2% H_2SO_4–acetone at a flow rate of 5 mL/min was sufficient to recover 88–91% of the retained picrate.

To achieve the brightest color for quantification, as much acetone as possible should be used with the minimum water to drive the ionization and subsequent color change. The absorbance of picrate in acetone is highly dependent on the water content (Fig. 5). Once sufficient water is present, additional water only dilutes the sample and reduces the color. However, the amount of water that gives the highest absorbance is in a very narrow peak of the curve. Precise measurement would then be critical to duplicate the predicted

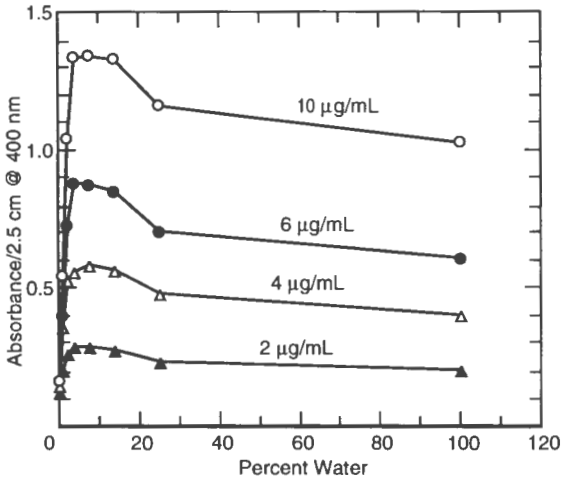

Figure 5. Dependence of picrate absorbance on water content of acetone.

calibration. A water content of 25% will give a reasonable value while being at the least sensitive part of the curve. Thus, small measurement errors that may occur under adverse field conditions would not have large effects on calculated results.

Potential interferences

Three environmental transformation products of TNT that produce yellow acetone extracts are 3,5-DNA, 2ADNT and 4ADNT. Maximum reported concentrations of these chemicals in explosives-contaminated soils (373 µg/g for 2ADNT and 14 µg/g for 3,5-DNA) were obtained from a report documenting analytical results for a number of soil samples from explosives-contaminated sites in the U.S. (Walsh et al. 1993). When these chemicals in an acetone–water solution at concentrations above the maximum reported levels were extracted with an Alumina-A cartridge, the yellow color was retained on the sorbant. A rinse with 3 mL of methanol removed all traces of the color. An additional rinse with 3 mL of acetone removed the methanol and returned the cartridge to the original extraction conditions, ready for the elution step. When subsequent eluents were analyzed by RP-HPLC, none of these analytes were detected. Thus these compounds do not interfere in this method.

Another source of yellow in acetone soil extracts is elemental sulfur.* The yellow color from a sulfurous acetone extract from an anaerobic Eagle River Flats, Alaska, sediment was not retained on Alumina-A.

The presence of humic materials in soil extracts was found to be a problem because these compounds are also highly colored acids which are retained by acidic ion exchangers. Some humic substances were eluted along with the picrate. The quantity of eluted humic color was highly variable (from 14 to 45% of the applied material) and absorbed strongly in the 400-nm region, where picrate absorbance is at its maximum for the HACH spectrophotometer (Fig. 6). However, since the development of the picrate color requires a dilution of the eluent with water, a background correction scheme was used. As previously optimized, 3.3 mL of water was needed to maximize the yellow color of the diluted 10 mL of eluent (producing a 13.3-mL mixture of 25% aqueous–acetone). This dilution would reduce the absorbance of the

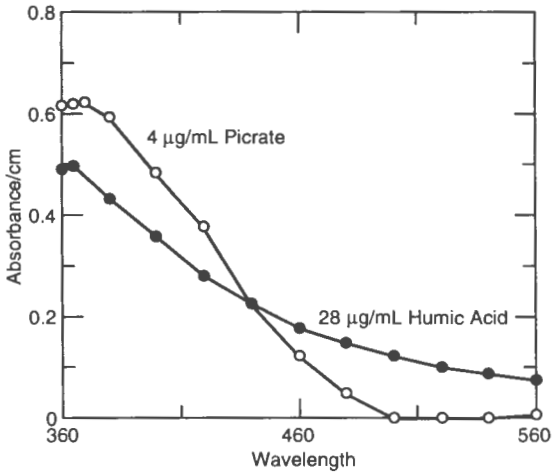

Figure 6. Absorbance curves for humic acid and picrate ion in 75% acetone–H_2SO_4.

humic materials by 25%. It was decided that a 50% reduction in interfering absorbances would produce a more reliable correction factor. This could be achieved by adding 5 mL of unacidified acetone followed by 5 mL of water to the 10 mL of acidic eluent (producing a 20-mL mixture of 25% aqueous acetone). The absorbance of the initial 10 mL of eluent is divided by the factor-of-two dilution and subtracted from the final absorbance of the 20 mL of acetone–water mixture.

This background scheme also provides an important confirmation step to the method. The addition of 5 mL of unacidified acetone will cause a visible decrease in the absorbance of colored eluents. The subsequent addition of 5 mL of water will then cause a visible increase in yellow if picrate is present.

Method detection limit

To establish the method detection limit (MDL) (USEPA 1984) for this method, a locally acquired sand was fortified by adding aqueous picrate solutions to the air-dried soil such that the resulting moisture content was 10%. A standard curve was constructed by processing 22-g samples that had been fortified with a range of picrate concentrations plus a blank sample. The curve that was calculated using a standard regression model had a very small, non-zero intercept. If absorbance readings were rounded to the nearest 0.01, this intercept became insignificant. Resulting picrate values should be reported using two significant digits. The quantity of picric acid based on a dry weight of 20 g of soil, extracted with 100 mL of acetone and quantified with a 1-cm path-length cell, was:

* M.E. Walsh, U.S. Army Cold Regions Research and Engineering Laboratory, personal communication, 1994.

picric acid (μg/g) = 91 (μg/g-ABS unit)
× (Final ABS @400 nm – 0.5
× Initial ABS @ 400 nm) (Fig. 7).

The computation of the MDL was based on the replicate extractions of seven 22-g samples of locally acquired sandy loam that had been fortified with 5 μg/g of picric acid. After a few minutes settling time, 30 mL of the acetone extract was filtered using a 0.45-μm Millex SR syringe filter, then diluted with 30 mL of reagent-grade water. The diluted extract was then passed through an Alumina-A cartridge using a 60-mL syringe at 10 mL/min. The cartridge was rinsed with 3 mL of methanol followed by 3 mL of acetone using a 10-mL syringe. A 10-mL aliquot of 2% H_2SO_4–acetone was then passed through the cartridge at 5 mL/min using a 10-mL syringe. The eluent was collected through another 0.45-μm Millex-SR syringe filter attached to the tip of the cartridge. The initial absorbance at 400 nm was recorded. An additional 5-mL aliquot of unacidified acetone was added to the extract. This dilution resulted in a visible decrease in the yellowish humus-derived color of the initial extract. Then 5 mL of reagent-grade water was added and the resulting deeper yellow color measured at 400 nm. The resulting MDL was 1.3 μg/g.

A field screening method that includes a quantification step requires a daily check standard for validation. An aqueous, unacidified standard at 10 μg/mL of picric acid is stable for at least two weeks. A simulated soil extract is prepared by diluting a 30-mL aliquot of this standard one-to-one with acetone. This mixture is then extracted and eluted according to the method. The absorbance/cm at 400 nm should be 0.56 ± 0.08.

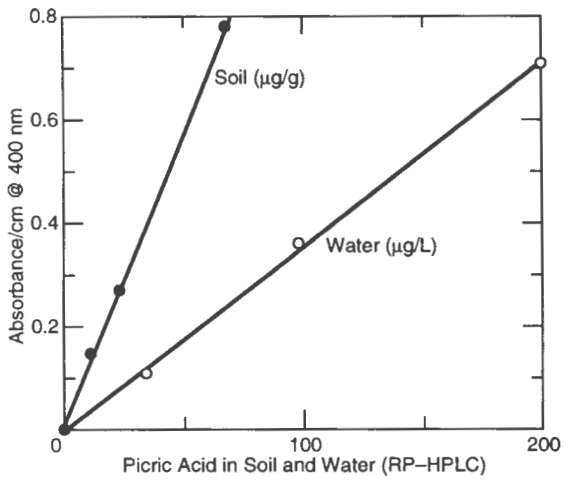

Figure 7. Calibration curves for soil and water methods.

Field screening in water

Selection of ion-exchange material and extraction and elution conditions

Initial tests with the basic ion-exchange materials indicated that the Anion membrane retained picrate from water but the Alumina-A cartridge did not. Thus development of the screening method for water utilized the Anion membrane.

A 2-L water sample fortified with 2 μg/L of picric acid was extracted with the Anion membrane in 20 minutes. The membrane turned yellow. Microscopic examination of a cross section of the membrane showed that all of the picrate was retained on the top surface of the membrane. One 5-mL aliquot of 10% H_2SO_4–methanol was added to the extraction apparatus and allowed to soak the membrane for three minutes. Vacuum was applied and the extraction solution pulled into a test tube placed beneath the membrane holder. This procedure removed most of the yellow color from the membrane, producing a colorless solution of undissociated picric acid. Some picrate remained in the ring of membrane that was clamped between the top and bottom of the extraction apparatus. This could be recovered by removing the reservoir top and pouring a few milliliters of extraction solution on the surface of the membrane, allowing that to soak, then pulling it through the membrane by vacuum. This procedure required some care and did not appear to be worthwhile for a field method. The additional recovery was in a more dilute solution than the original 5 mL of extract, reducing the overall absorptivity of the combined extracts. An addition of 5 mL of reagent-grade water produced the maximum yellow color from the picrate ion.

A breakthrough test was performed using reagent-grade water fortified with 20,000 μg/L of picric acid. The run-through was measured using RP-HPLC. No breakthrough was observed when 2 L of water at this concentration was extracted.

Potential interferences

The same yellow TNT transformation products that were retained by the Alumina-A (two isomers of amino-DNT and 3,5-DNA) were also retained by the Anion membrane. A 5-mL methanol wash removed these from the membrane without eluting any picrate.

Humic acids produced the same problems in the water method as in the soil method. Humic material was retained by the Anion membrane, then eluted by the acidified solvent. Fortunately

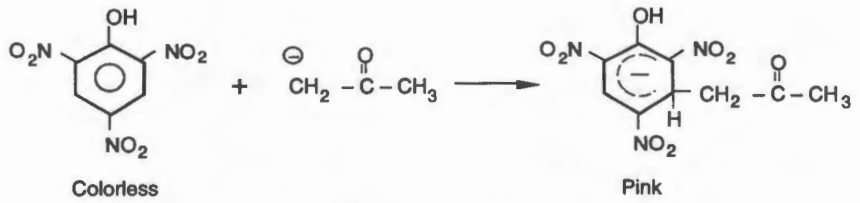

Figure 8. Formation of the colored Meisenheimer anion from picric acid.

the required 50% dilution of the 5-mL eluent provided a background correction factor similar to the soil method.

Qualitative confirmation of picrate

The extraction of 2 L of reagent-grade water fortified with 2 µg/L of picrate produced a visibly yellow surface on the Anion membrane. A lack of any yellow color on the membrane is an indication that less than 2 µg/L of picrate is present in the sample. If there is a yellow color remaining after the methanol rinse, it could be picrate at a level above 2 µg/L. Since picric acid is a nitroaromatic, it forms a colored Meisenheimer anion when exposed to the Janowsky conditions of a basic ketone solution (Kabeya et al. 1973) (Fig. 8). Experiments were performed to see if this reaction would occur with the sorbed picrate. A quaternary ammonium salt reagent commercially available as part of the EnSys TNT detection kit was used to confirm the presence of picric acid on the surface of the Anion membrane, both with and without brown humic interferences. An additional acetone rinse was added after the methanol rinse. One or two drops of EnSys reagent was then applied and the color observed. When the Anion membrane was yellow or brown, the Ensys reagent turned a noticeable pink or dark rust, respectively, confirming that the yellow color was due to a nitroaromatic.

This colorimetric confirmation scheme was tried for the soil method; however, in the cartridges the yellow was dispersed throughout a few millimeters of the sorbant bed so that the pink that was produced was barely discernible, even when there were no brown interferences. The presence of any brown completely obscured the pink EnSys color in the cartridge.

Method detection limit

A standard curve was constructed by making a series of 2-L solutions of reagent-grade water fortified with picric acid, plus a blank. The calculated curve that resulted had a small non-zero intercept similar to the curve calculated for the soil method. This became insignificant when absorbances were rounded to the nearest 0.01. Concentrations of picric acid should be reported using two significant digits. For a 2-L water sample and a spectrophotometer with a 1-cm path-length cell:

picric acid (µg/L) = 280 (µg/L-ABS unit)
× (Final ABS @ 400 nm – 0.5
× Initial ABS @ 400 nm) (Fig. 7).

To estimate the MDL, a series of seven replicate 2-L well-water samples fortified with 7.5 µg/L of picric acid were extracted, rinsed with 5 mL of methanol, then eluted with 5 mL of 10% H_2SO_4–methanol and the absorbance at 400 nm of the resulting extract recorded. The extract was diluted with 5 mL of reagent-grade water and the absorbance at 400 nm of the diluted extract obtained. The MDL was 3.6 µg/L.

A daily calibration standard is made by diluting 30 mL of a 10 µg/mL aqueous solution of picric acid to 2 L with reagent-grade water and performing the method. The absorbance/cm at 400 nm should be 0.56 ± 0.03.

Performance evaluations

Soil method

The method was tested on field-contaminated soils. The results are listed in Table 2. Soils from Crane, Indiana, produced "straw-colored" acetone extracts. Analyses by HPLC showed that they contained no picrate. The field screening method produced a very light yellow Alumina-A extract that was reduced by dilution. The soil from Hawthorne required a 360-fold dilution to fall within the linear range of the calibration curve. The soils from Mead had been analyzed previously by Method 8330. The only detected analyte had been tetryl. Both the field screening method and the HPLC method using the buffered eluent system revealed the presence of picrate. Since picrate is a hydrolysis product of tetryl (Kayser et al. 1984, Kayser

Table 2. Comparison of picric acid determinations between field screening method and HPLC.

Sample	*Picric acid screening (μg/g)*	*Picric acid HPLC (μg/g)*	*Tetryl HPLC (μg/g)*
Crane162	0.0	0.0	0.0
Crane541	0.0	0.0	0.0
Hawthorne13	25,200	23,000	0.0
Mead42	1.9	1.0	60
Mead43	14.0	24.4	1265
Mead45	6.8	9.8	397

and Burlinson 1988, Jenkins et al. 1995), it is to be expected in environmental samples contaminated with tetryl.

Water method

The MDL determinations were performed using fortified well waters, which produced a slightly brown acidic extract. As a worst-case example of background interference due to dissolved humic substances, this method was tested on river water that had been fortified with 125 μg/L of tetryl and allowed to sit at room temperature in the dark for 60 days. HPLC analysis showed that 64% of the tetryl had degraded, producing 25 μg/L of picrate plus several other unidentified products. The Anion membrane turned a dirty brown color when this sample was extracted. Application of a drop of EnSys reagent did produce a rusty colored spot. Elution with 5 mL of 10% H_2SO_4–methanol produced a dark yellow-brown extract with an initial absorbance at 400 nm of 1.3. The subsequent dilution with reagent-grade water and the final absorbance reading resulted in a calculated concentration of 39 μg/L of picric acid.

Water samples from 34 monitoring wells at the Naval Surface Warfare Center in Crane, Indiana, were screened for picrate using the proposed method. Since there was only one 500-mL sample available from each well, samples were composited to make 2-L samples for extraction. All composites had been previously extracted for nonionic explosives using Empore SDB extraction membranes, which do not retain picrate. The samples at that point were colorless and free of sediment. All the composites produced pink- or salmon-colored deposits on the Anion membranes. The addition of EnSys reagent did not produce a darker spot. These deposits were removed by the acidified methanol, producing light yellow extracts whose colors were reduced upon dilution with water. A spectrophotometer was not used for quantification; however, the lack of a visible increase in color upon addition of water indicated that little or no picrate was present. Subsequent RP-HPLC analysis confirmed the absence of picrate in all of the samples.

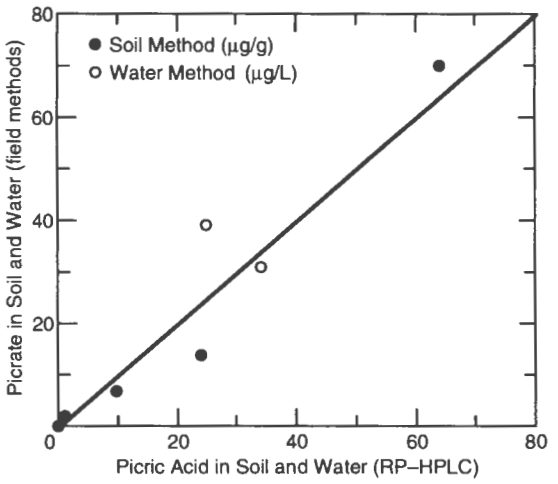

Figure 9. Comparison between field methods and RP-HPLC determination of picrate.

A comparison between field method and RP-HPLC determinations of picrate in contaminated or fortified environmental matrices shows reasonable agreement (Fig. 9). Rigorous statistical evaluations can be made after more field samples are analyzed. Future tests of the water method will have to be conducted as part of a sampling plan that considers the 2-L sample requirement.

Coordination with existing methods

The soil method can be easily added to accepted screening methods for the military explosives TNT and RDX in soil (Jenkins and Walsh 1992). A single 100-mL acetone extract can be split for each of the three tests. The Alumina-A cartridges are required for the RDX test to remove interfering nitrates and nitrites.

A field screening method for TNT and RDX in water that uses Empore SDB membranes has been proposed (Jenkins et al. 1994). Both that method and the one proposed in this report require a vacuum filtration apparatus. A hand-operated vacuum pump can be used, but it requires constant pumping for at least 20 minutes for sediment-free samples. If sediment causes partial plugging of the membrane, the hand pump is inadequate. Realistically, a powered vacuum pump is required to supply sufficient suction to extract 2-L samples in a reasonable time.

Table 3. Cross-reactivities of commercial TNT immunoassays.

	Millipore	*D-TECH*
Tetryl	–	+
2Am-DNT	–	+
4Am-DNT	+	–
TNB	+	+
2,6-DNT	+	–
2,4-DNT	–	+

IMMUNOASSAYS FOR PICRATE IN SOIL AND WATER

Immunoassays are available commercially for detecting TNT in soil extracts and water. A disadvantage of all immunoassays is that they also detect compounds that are structurally related to the target analyte. This phenomenon is called cross-reactivity. Antibodies are produced in response to small molecules (molecular weight less than 200) only after they have been conjugated to a large carrier protein. Exactly how this conjugate is made determines the sensitivity of the assay and the degree of cross-reactivity to various compounds (Harrison et al. 1991). In the case of TNT, conjugates could be made by coupling a protein to either a reactive moiety at the 1- position (e.g. trinitro-sulphonic acid) or at the 2- or 4- position (2- or 4-aminodinitrotoluene). The antibody would then tend to recognize either a trinitro-aromatic or a dinitro-toluene, respectively. Judging from the cross-reactivities listed on two commercial TNT kits (Table 3), it was assumed that they were produced using these different schemes. A test of their cross-reactivies to picrate showed that the EnviroGard TNT plate kit was mildly responsive, with a detection limit of about 2.5 µg/g in soil and 5 µg/L in water (the detection limit of TNT is 0.25 µg/g in soil and 0.5 µg/L in water). The D-TECH kit was equally sensitive to TNT and picrate, with detection limits of 0.2 µg/g in soil and 5 µg/L in water.

CONCLUSIONS

The proposed methods (App. A and B) for field screening for residues of picric acid or ammonium picrate in soil and water resulted from the combined application of contemporary solid-phase extraction materials with a 70-year-old, qualitative colorimetric assay. The chemical confirmation reagents used were also derived from work done by chemists around the turn of the century (reviewed in Jenkins 1990). The resulting methods are both sensitive and relatively free from interferences produced by humic substances or other nitroaromatics that are likely to occur at military sites. A single extract can be used to screen for picric acid, ammonium picrate, TNT, 2,4DNT and RDX in soils. The estimated cost of a few dollars per sample is very low. Single assays can be run in about 20 minutes. Multiple samples can be processed in less time using cartridge or membrane manifolds.

Immunoassays are gaining increased acceptance in screening applications, although the costs and time requirements can be significantly higher than spectrophotometric methods. The positive response to picric acid of two TNT immunoassays presents two interesting possibilities. When a field site is known to contain no nitroaromatics other than picric acid or ammonium picrate, immunoassays could be used to screen for picrate. Although this may be unusual at military sites, it should be the case at industrial sites contaminated with picric acid. Conversely, if a TNT immunoassay gives a response that is greater than the sum of TNT and other known cross-reactive analytes as determined by Method 8330, unsuspected contamination by picric acid may be indicated.

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APPENDIX A: METHOD DOCUMENTATION: FIELD METHOD FOR DETERMINATION OF AMMONIUM PICRATE AND PICRIC ACID IN SOIL

1.0 SCOPE AND APPLICATION

This method is suitable for the determination of ammonium picrate and picric acid in field-moist or dried soil, using a battery-operated spectrophotometer.

2.0 SUMMARY OF METHOD

A 20-g subsample of soil is extracted with 100 mL of acetone by manually shaking for three minutes. A 30-mL aliquot is filtered into a graduated cylinder and diluted with 30 mL of deionized water. This converts picric acid or ammonium picrate in the extract to picrate ions. The diluted extract is then passed through an ion-exchange solid-phase extraction (SPE) tube. Picrate ions are retained on the resin. Interferences are removed by washing the resin with methanol. Picrate ions are converted to protonated picric acid and eluted from the resin by washing with 10 mL of acetone containing four drops of concentrated sulfuric acid. The initial absorbance of the eluent at 400 nm is recorded and provides a background correction. The colorless, protonated picric acid solution is converted to a yellow picrate ion solution by diluting the acidified acetone extract with water. The absorbance is measured again at 400 nm. The corrected absorbance is converted to µg/g of picric acid based on the response from calibration standards.

3.0 DEFINITIONS

Ammonium picrate (ammonium 2,4,6-trinitrophenoxide)
MW: 246.14
Solubility in water at 25°C: >10 g/L
Octanol–water partition coefficient: 0.2
CAS# 131-74-8

Picric acid (2,4,6-trinitrophenol)
MW: 229.11
Solubility in water at 25°C: >10 g/L
Octanol–water partition coefficient: 4.4
CAS# 88-89-1

4.0 INTERFERENCES

Identification and quantification is based on the absorbance of picrate ions at 400 nm. Other substances that can produce yellow extracts include humic materials and certain nitroaromatic secondary explosives and their environmental transformation products. Extraction with ion-exchange resins followed by a rinse with methanol removes the nitroaromatic interferences. Background correction using the initial absorbance of the acid–acetone eluent accounts for humic materials. Rinsing the spectrophotometer cuvette with acetone between samples is necessary. Carry-over of any water from the previous water–acid–acetone mixture will convert colorless, protonated picric acid in the acid–acetone mixture to yellow picrate ions. This will invalidate the "Initial Absorbance" measurement that is required for background correction.

5.0 SAFETY

The normal safety precautions associated with the use of flammable organic solvents, strong acids and potentially toxic chemicals should be employed. Eye protection is recommended when shaking bottles to protect against splash from poorly sealed containers. Eye, hand and clothing protection is recommended when handling the concentrated sulfuric acid and acidified eluents.

6.0 EQUIPMENT AND SUPPLIES

A. Instrumentation:
   1. Field-portable, battery-operated spectrophotometer with a 1-cm path-length cell (Hach DR2000 or equivalent).
   2. Mechanical or battery-operated balance to measure soils.
   3. Analytical balance for preparation of standards.

B. Labware and equipment
   1. 250-mL (Nalgene or equivalent) plastic bottles with caps containing three 1/4-inch steel ball bearings, one per sample.

2. 120-mL (Nalgene or equivalent) plastic bottles with caps, five for calibration solutions.
3. Volumetric pipets: one each—10, 8, 6, 4, 2 mL.
4. Volumetric flasks: 100-mL (two).
5. 100-mL graduated cylinder.
6. 10-mL graduated cylinders (two).
7. Disposable eyedroppers or pasteur pipets (one for sulfuric acid and one for each sample).
8. Millex SR filter units, 0.5 µm, two per sample.
9. Glass cuvettes, 1-cm path length.
10. Disposable plastic syringes: 60-mL (one per sample), 10-mL (two per day for acidified acetone and rinses).
11. 22-mL glass vials with caps (one per sample).
12. Supelclean Alumina-A, solid-phase extraction (SPE) tubes, 3 mL (Supelco #5-7092) (one per sample).
13. Teflon-coated stir bar (10 × 15 mm).
14. 500-mL squirt bottles (three).
15. Bottle-top dispenser for acetone (highly recommended).
16. Timer or wristwatch to measure flow rates.

7.0 REAGENTS AND STANDARDS

1. Picric acid (SARM or reagent grade).
2. Acetone, commercial grade.
3. Methanol, reagent grade.
4. Concentrated sulfuric acid, reagent grade.
5. Water, deionized.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

This method may be used with field-moist or dried soil samples.

A soil sample is mixed as thoroughly as possible and a 20-g subsample added to a 250-mL plastic bottle containing three 1/4-inch steel ball bearings. The bottle is capped until extraction is conducted. The samples should be keep cold (4°C) and in the dark until extraction takes place. Samples should be analyzed the same day they are collected.

9.0 QUALITY CONTROL

9.1 GENERAL: The accuracy and precision of this method are subject to the common errors associated with poor-quality measurements of weights and volumes. There are a few sources of error specific to this method. The binding and release of picrate in the solid-phase extraction (SPE) tube is flow-rate dependent. The flow rates specified in the method (Procedure Steps 5 and 10) should be implemented carefully. The removal of residual water from the spectrophotometer cuvettes (Procedure Step 17) is critical for successful application of the background correction calculation. The manufacturer's instructions for the SPE tubes call for a conditioning rinse. This step is not necessary. The tubes may be used directly from the package.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE: An initial calibration curve for the method should be performed as directed in CALIBRATION AND STANDARDIZATION. The calibration curve should be linear, with a zero intercept and a slope (response factor) of 90 ± 15. The linear range of the method is 0.0–69 µg/g.

9.3 ASSESSING PERFORMANCE: A method detection limit (MDL) analysis should be performed. A 150-g sample of blank soil is spiked with 6.0 mL of the 125-mg/L working standard to produce a picric acid concentration of 5 µg/g. The soil is homogenized as completely as possible. Seven 20-g subsamples are processed according to the method. The concentration of picric acid in the subsamples is determined using the initial calibration curve. The standard deviation (SD) of the seven determinations is calculated. The MDL = 3.14 × SD and should be about 1.3 µg/g.

The recovery of the spiked picric acid should be between 75 and 125%.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY: Blank and spiked soil samples should be analyzed daily as directed in CALIBRATION AND STANDARDIZATION and records kept. The background-corrected absorbance of a 50-µg/g soil spike should be above 85% of the value of the 10-µg/L calibration solution processed according to the directions for the daily calibration of response factor. The blank soil should produce a background-corrected absorbance less than 0.01 absorbance units. The relative standard deviation of daily matrix spikes should be less than 15%.

10.0 CALIBRATION AND STANDARDIZATION

10.1 PREPARATION OF STANDARDS: About 0.2 g of solid picric acid (SARM or reagent grade) is dried to constant weight in a vacuum desiccator in the dark. About 0.125 g is weighed out to the nearest 0.1 mg, transferred to a 100-mL volumetric flask and diluted to volume with deionized water. A stir bar is added and the solution stirred overnight in the dark. The concentration of picric acid in this stock is about 1250 mg/L. A working standard is made by diluting 10 mL of the stock with deionized water to 100 mL in a volumetric flask. The concentration of the working standard is about 125 mg/L.

Calibration solutions are prepared in 125-mL plastic bottles by diluting the working standard with deionized water as described in Table A1. Volumetric pipets are used to measure the working standard. A 100-mL graduated cylinder is used to measure the dilution water.

10.2 INITIAL CALIBRATION: A 30-mL aliquot of each aqueous calibration solution is diluted with 30 mL of acetone and processed according to the PROCEDURE starting at Step 5. Absorbance readings of Solutions A-E should range from 0.0 to 0.7 absorbance units. The slope of the relationship between the concentration of picric acid and absorbance should be linear, with a zero intercept and a response factor of 90 ± 15 µg/g per absorbance unit on a wet-weight basis.

10.3 DAILY CALIBRATION: A 30-mL aliquot of Calibration Solution D (10 µg/mL, equivalent to 50 µg/g in soil) is diluted with 30 mL of acetone and processed according to the method. The daily response factor is calculated:

daily response factor = (50 µg/g)/
(Absorbance @ 400 nm).

10.4 DAILY BLANK AND MATRIX SPIKE: A blank soil is produced by adding a 100-mL aliquot of acetone to 20 g of a soil known to be uncontaminated with picrate and performing the method. A soil spiked at a concentration of 50 µg/g is made by adding 8 mL of the 125-mg/L working standard to 20 g of blank soil followed by 92 mL of acetone and performing the method.

11.0 PROCEDURE

1. A 100-mL aliquot of acetone is measured in a graduated cylinder and added to the 250-mL sample bottle containing 20 g of soil. The bottle is capped and manually shaken for three minutes.
2. The soil is allowed to settle for 5 minutes.
3. The sample bottle cap is removed and a 60-mL syringe is inserted into the extract. About 35–40 mL of extract is pulled into the syringe. A Millex SR filter is placed on the tip of the syringe and 30 mL of extract is expressed into a 100-mL graduated cylinder. Any remaining extract can be filtered back into the extraction bottle. The filter is removed and placed in a waste container.
4. The 30 mL of acetone extract is diluted in the 100-mL graduated cylinder to 60 mL with deionized water. This assures that all the picric acid and ammonium picrate in the acetone extract is converted to picrate ion.
5. A 3-mL Alumina-A solid-phase extraction (SPE) tube is placed on the end on the same 60-mL syringe with the plunger removed. The aqueous–acetone mixture is added to the syringe and the plunger replaced. The plunger is depressed so that the mixture flows through the SPE tube at about 10 mL/minute. The picrate ions are retained in the SPE tube. The eluent is collected in a waste container.
6. The 60-mL syringe is removed from the SPE tube and placed in a waste container.
7. The SPE tube is filled with methanol from a squirt bottle (about 3 mL) and an air-filled 10-mL syringe is used to force the methanol through the tube into the waste container. The SPE tube is then filled with acetone from a squirt bottle (about 3 mL) and rinsed as above into the waste container.
8. The SPE tube is prepared for elution of picric acid by placing a new Millex SR filter on the tip and placing a plungerless 10-mL syringe in the barrel.
9. A 10-ml aliquot of acetone is measured in one 10-mL graduated cylinder. Four drops of concentrated sulfuric acid are added to the acetone using an eyedropper. The tip of the dropper should be close to the surface of the acetone to prevent splashing from the falling drop.
10. The 10-mL acid–acetone mixture is poured into a 10-mL syringe and the plunger replaced while the tip of the extraction tube–filter assem-

bly is held in the mouth of a 22-mL glass vial. The plunger is depressed so that the mixture passes through the SPE tube at about 5 mL/minute and is collected in the glass vial. The acid–acetone mixture converts the retained picrate ions to unretained, protonated picric acid. The SPE tube and filter are placed in a waste container.

11. A portion of the acid–acetone eluent is transferred with a disposable pasteur pipet to a glass spectrophotometer cuvette and the absorbance at 400 nm measured and recorded as Initial ABS. The spectrophotometer is zeroed with water.

12. The eluent is returned to the 22-mL glass vial and the cuvette rinsed with water from a squirt bottle and discarded.

13. If the eluent has any color, a qualitative description is recorded. A 5-mL aliquot of unacidified acetone plus a 5-mL aliquot of water is measured in a second 10-mL graduated cylinder and added to the 22-mL vial. This converts any colorless, protonated picric acid to yellow picrate ions.

14. A portion of the diluted eluent is transferred to the cuvette using the same disposable pasteur pipet and the absorbance at 400 nm measured and recorded as Final ABS.

15. If the Final ABS is greater than 0.7, the diluted eluent may be diluted further with water and remeasured.

16. After measurement, the eluent is returned to the 22-mL vial and the pipet is placed in a waste container.

17. The cuvette is rinsed, first with water from a squirt bottle, then with acetone from a squirt bottle. It is critical that no residual water be present in the cuvette when the next sample is added to the cuvette for the Initial ABS measurement. Water will ionize any picric acid to picrate and invalidate the background correction.

12. DATA ANALYSIS AND CALCULATIONS

The absorbance data are converted to picric acid concentration in the sample by the following formula:

$$\mu g/g \text{ picric acid} = \text{daily response factor } (\mu g/g\text{–ABS unit}) \times (\text{Final ABS} - 0.5 \times \text{Initial ABS}).$$

This is based on a 20-g wet-weight subsample extracted with 100 mL of acetone and measured using the dilutions specified in the Procedure. Any dilution that was required to bring the eluent into the linear range of the initial calibration should be included in the calculations using the following formula:

$$\mu g/g \text{ picric acid} = \text{daily } rf \ (\mu g/g\text{-ABS unit}) \times [(\text{Final ABS} \times df) - 0.5 \times (\text{Initial ABS}/df)]$$

where rf is the response factor and df is the dilution factor.

13. METHOD PERFORMANCE

The method has been applied to a series of soil samples whose picric acid concentrations had been determined by RP-HPLC (Table A2).

14. POLLUTION PREVENTION

All containers of organic solvents and extraction solutions should be kept capped to prevent evaporation. A bottle-top dispenser is highly recommended to prevent acetone spillage. Alternatively, a large, deep funnel may be used when measuring the acetone into the 100-mL graduated cylinder. A large tray should be used under the work area to contain any spilled solvents.

15. WASTE MANAGEMENT

All solid waste contaminated with solvents, acid and extracted chemicals should be disposed of according to Federal, state and local regulations. This includes extraction bottles and soils, filters, syringes, disposable pipets, SPE tubes and eluent vials.

All waste organic solvents from the extraction procedures and rinses should be disposed of according to Federal, state and local regulations.

All acid–solvent mixtures should be treated separately as required by Federal, state and local regulations.

16. REFERENCE

Thorne, P.G. and T.F. Jenkins, "Development of a Field Method for Ammonium Picrate and Picric Acid in Soil and Water," Special Report 95-20, USA Cold Regions Research and Engineering Laboratory, Hanover, N.H.

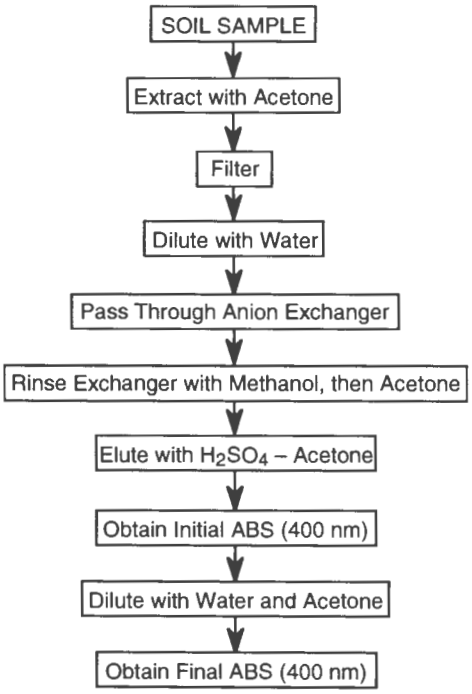
17. TABLES AND FLOW DIAGRAM

Table A1. Preparation of calibration solutions from 125-mg/L working standard to simulate extracts of 20-g soil subsamples with 100 mL of acetone.

Solution	*Volume of working STD (mL)*	*Volume of water (mL)*	*Conc. (µg/mL)*	*Associated soil conc. (µg/g)*
A	0	100	0.0	0.0
B	2	98	2.5	12.5
C	4	96	5.0	25.0
D	8	92	10.0	50.0
E	10	90	12.5	62.5

Table A2. Comparison of picric acid determinations between field screening method and HPLC.

	Picric acid (µg/g)	
Sample	*Screening*	*HPLC*
1	0.0	0.0
2	0.0	0.0
3	25,200	23,000
4	1.9	1.0
5	14.0	24.4
6	6.8	9.8



APPENDIX B: METHOD DOCUMENTATION FIELD METHOD FOR DETERMINATION OF AMMONIUM PICRATE AND PICRIC ACID IN WATER

1.0 SCOPE AND APPLICATION

This method is suitable for the determination of ammonium picrate and picric acid in well water or surface water. A battery-operated spectrophotometer and hand vacuum pump can be used; however, a powered vacuum source is recommended.

2.0 SUMMARY OF METHOD

A 2-L subsample of water is drawn through an anion extraction disk under vacuum. Picrate ions are retained by the disk. Interferences are removed by washing the disk with methanol. Picrate ions are converted to protonated picric acid and eluted from the disk using a mixture of 4.5 mL of methanol plus 0.5 mL of concentrated sulfuric acid. The initial absorbance of the eluent at 400 nm is recorded and provides a background correction. The eluted colorless, protonated picric acid is converted to a yellow picrate ion solution by dilution with water. The absorbance is measured again at 400 nm. The background-corrected absorbance is converted to µg/L of picric acid based on the response from calibration standards.

3.0 DEFINITIONS

Ammonium picrate (ammonium 2,4,6-trinitrophenoxide)
MW: 246.14
Solubility in water at 25°C: >10 g/L
Octanol–water partition coefficient: 0.2
CAS# 131-74-8

Picric acid (2,4,6-trinitrophenol)
MW: 229.11
Solubility in water at 25°C: >10 g/L
Octanol–water partition coefficient: 4.4
CAS# 88-89-1

4.0 INTERFERENCES

Identification and quantification is based on the absorbance of picrate ions at 400 nm. Other substances that can produce yellow extracts include humic materials and certain nitroaromatic secondary explosives and their environmental transformation products. Extraction with ion-exchange resins followed by a rinse with methanol removes the nitroaromtic interferences. Background correction using the initial absorbance of the acid–methanol eluent accounts for humic materials. Rinsing the spectrophotometer cuvette with methanol between samples is necessary. Carryover of any water from the previous water–acid–methanol mixture will convert colorless, protonated picric acid in the acid–methanol mixture to yellow picrate ions. This will invalidate the "Initial Absorbance" measurement that is required for background correction.

5.0 SAFETY

The normal safety precautions associated with the use of flammable organic solvents, strong acids and potentially toxic chemicals should be employed. Eye, hand and clothing protection is recommended when handling the concentrated sulfuric acid and acidified eluents.

6.0 EQUIPMENT AND SUPPLIES

A. Instrumentation:
1. Field-portable, battery-operated spectrophotometer with a 1-cm path-length cell (Hach DR2000 or equivalent).
2. Analytical balance for preparation of standards.
3. Vacuum source.

B. Labware and Equipment
1. 1-L (Qorpak or equivalent) amber glass sample bottles (two per sample).

2. 120-mL (Nalgene or equivalent) plastic bottle with cap (one for working standard).
3. Volumetric pipets: 10, 8, 5, 2 mL.
4. Volumetric flasks: 100-mL (one), 1 L (one).
5. 2-L graduated cylinder.
6. 10-mL graduated cylinder (two).
7. Disposable eyedropper (for sulfuric acid).
8. Glass fiber filters, 47 mm (for sediment-laden samples).
9. Glass cuvettes, 1-cm path length (two).
10. Vacuum filtration apparatus, 1-L vacuum flask with 47-mm filter holder and funnel.
11. 24- × 200-mm glass test tubes with screw-caps (one per sample).
12. Empore Anion Extraction Disks, 47-mm (Varian).
13. Pasteur pipets, 9 inch (one per sample).
14. 500-mL squirt bottles (two).

7.0 REAGENT AND STANDARDS

1. Picric acid (SARM or reagent grade).
2. Methanol, reagent grade.
3. Concentrated sulfuric acid, reagent grade.
4. Water, deionized.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

This method may be used with well water or surface water.

A water sample is collected in two 1-L amber glass bottles which are capped and kept cold (4°C) in the dark until extraction. Samples should be analyzed the same day they are collected.

9.0 QUALITY CONTROL

9.1 GENERAL: The accuracy and precision of this method are subject to the common errors associated with poor-quality measurements of weights and volumes. There is a source of error specific to this method. The removal of residual water from the spectrophotometer cuvettes (PROCEDURE Step 17) is critical for successful application of the background correction calculation. The manufacturer's instructions for the extraction disks call for a conditioning rinse. This step is not necessary. The disks may be used directly from the package.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE: An initial calibration curve for the method should be developed as directed in CALIBRATION AND STANDARDIZATION. The calibration curve should be linear, with a zero intercept and a slope (response factor) of 280 ± 75. The linear range of the method is 0.0–200 µg/L.

9.3 ASSESSING PERFORMANCE: A method detection limit (MDL) analysis should be performed. A 20-L sample of deionized water is spiked with 20 mL of the 10-mg/L working standard to produce a picric acid concentration of 10 µg/L. The water is stirred as completely as possible. Seven 2-L subsamples are processed according to the method. The concentration of picric acid in the subsamples is determined using the initial calibration curve. The standard deviation (SD) of the seven determinations is calculated. The MDL = 3.14 × SD and should be about 3.6 µg/L.

The recovery of the spiked picric acid should be between 75 and 125%.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY: Blank and spiked water samples should be analyzed daily as directed in CALIBRATION AND STANDARDIZATION and records kept. The background-corrected absorbance of a 150-µg/L water spike should be above 80% of the predicted absorbance based on the initial calibration curve. The blank water should produce a background-corrected absorbance less than 0.01 absorbance units. The relative standard deviation of daily matrix spikes should be less than 27%.

10. CALIBRATION AND STANDARDIZATION

10.1 PREPARATION OF STANDARDS: About 0.2 g of solid picric acid (SARM or reagent grade) is dried to constant weight in a vacuum desiccator in the dark. About 0.125 g is weighed out to the nearest 0.1 mg, transferred to a 100-mL volumetric flask and diluted to volume with deionized water. A stir bar is added and the solution stirred overnight in the dark. The concentration of picric acid in this stock is about 1250 mg/L. A working standard is made by diluting 8 mL of the stock with deionized water to 1 L in a volumetric flask. The concentration of the working standard is about 10 mg/L.

Calibration solutions are prepared in a 2-L graduated cylinder by diluting the working stan-

dard with deionized water as described in Table B1. Volumetric pipets are used to measure the working standard.

10.2 INITIAL CALIBRATION: Calibration solutions are prepared in the 2-L graduated cylinder and processed according to the PROCEDURE starting at Step 1. Absorbance readings of Solutions A–E should range from 0.0 to 0.7 absorbance units. The slope of the relationship between the concentration of picric acid and absorbance should be linear, with zero intercept and a response factor of about 280 µg/L per absorbance unit.

10.3 DAILY CALIBRATION: A 150-µg/L picric acid solution is made by diluting 30 mL of working standard to 2 L with deionized water and proceeding with the method. The daily response factor is then determined:

Daily response factor = (150 µg/L)/
(Absorbance @ 400 nm).

11. PROCEDURE

1. A 47-mm Empore Anion Extraction Disk is placed on a vacuum filter holder. A glass fiber filter may be placed on top of that if the sample appears cloudy. The funnel is clamped in place.

2. The water sample is measured in a 2-L graduated cylinder and a portion is added to the extraction funnel. The vacuum is applied and the funnel refilled as needed. After the first 1-L is extracted, the vacuum is shut off and vented and the vacuum flask emptied into a waste container. The vacuum is reapplied, the second liter extracted and the vacuum flask emptied again. Picrate ions in the sample are retained on the anion disk.

3. The glass fiber filter, if used, is removed and placed in a waste container. The funnel is reclamped in place. Air is drawn through the Anion disk for 5 minutes using vacuum.

4. The vacuum is turned off and vented, then 10 mL of methanol is added to the funnel. This is allowed to soak into the disk for 2 minutes, then pulled through with vacuum. This rinse removes interfering compounds.

5. The vacuum is vented and the extraction holder and funnel are removed so that the methanol rinse may be transferred to a waste container. A 25- × 200-mm test tube is placed into the vacuum flask. The extraction holder assembly is returned to the vacuum flask, with its drain tube inserted into the test tube.

6. A 4.5-mL aliquot of methanol is added to one 10-mL graduated cylinder. A 0.5-mL aliquot of concentrated sulfuric acid is added slowly with an eyedropper. The dropper should be held close to the surface of the methanol to prevent splashing.

7. The acid–methanol mixture is poured into the extraction funnel and allowed to soak the disk for 2 minutes. This converts the picrate ions to protonated picric acid.

8. Vacuum is applied and the acid–methanol eluent is collected in the test tube. The vacuum is vented and the test tube withdrawn and capped.

9. The Anion disk is removed from the holder and placed in a waste container. The holder and funnel are assembled and returned to the vacuum flask and rinsed with 10 mL of deionized water, which is collected in the vacuum flask and then transferred to a waste container.

10. A portion of the acid–methanol eluent is transferred from the test tube to a spectrophotometer cuvette using a 9-in. pipet.

11. The absorbance at 400 nm is measured and recorded as Initial ABS. The spectrophotometer is zeroed with water.

12. The eluent is returned to the test tube and the cuvette rinsed with water.

13. Any color in the eluent is qualitatively described and recorded. A 5.0-mL aliquot of deionized water is measured in a second 10-mL graduated cylinder and added to the 5.0 mL of acid–methanol eluent in the test tube.

14. Any change in color should be qualitatively described and recorded. An increase in yellow color confirms the presence of picrate in the sample.

15. A portion of the diluted eluent is transferred to the cuvette and the absorbance at 400 nm measured and recorded as Final ABS.

16. If the Final ABS is greater than 0.7, the diluted eluent may be diluted further with water and remeasured.

17. After measurement, the eluent is returned to the test tube.

18. The cuvette is then rinsed, first with water from a squirt bottle, then with unacidified methanol from a squirt bottle. It is critical that no residual water be present in the cuvette when the next sample is added to the cuvette for the Initial ABS measurement. Water will convert any picric acid to picrate ion and invalidate the background correction.

12. DATA ANALYSIS AND CALCULATIONS

The absorbance data are converted to picric acid concentration in the sample by the following formula:

$$\mu g/L \text{ picric acid} = \text{daily response factor } (\mu g/L\text{-ABS unit}) \times (\text{Final ABS} - 0.5 \times \text{Initial ABS}).$$

This is based on a 2-L subsample of water using the dilutions specified in the PROCEDURE. Any dilution that was required to bring the eluent into the linear range of the initial calibration should be included in the calculations using the following formula:

$$\mu g/L \text{ picric acid} = \text{daily } rf\ (\mu g/L\text{-ABS unit}) \times [(\text{Final ABS} \times df) - 0.5 \times (\text{Initial ABS}/df)]$$

where *rf* is the response factor and *df* is the dilution factor.

13. METHOD PERFORMANCE

The method was tested on 34 well waters. The field method indicated no picrate contamination above the detection limit. This result was confirmed using HPLC.

14. POLLUTION PREVENTION

All containers of organic solvents and extraction solutions should be keep capped to prevent evaporation. A large tray should be used under the work area to contain any spilled solvents.

15. WASTE MANAGEMENT

All solid waste contaminated with solvents, acid and extracted chemicals should be disposed of according to Federal, state and local regulations. This includes extraction disks, glass fiber filters, pasteur pipets and eluent test tubes.

All waste methanol from the rinses should be disposed of according to Federal, state and local regulations.

All acid–methanol mixtures should be treated separately as required by Federal, state and local regulations.

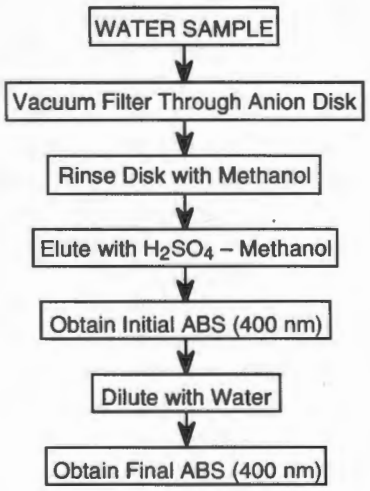
16. REFERENCES

Thorne, P.G. and T.F. Jenkins, "Development of a Field Method for Ammonium Picrate and Picric Acid in Soil and Water," Special Report 95-20, USA Cold Regions Research and Engineering Laboratory, Hanover, N.H.

17. TABLE AND FLOW DIAGRAM

TABLE B1. Preparation of calibration solutions from the 10-mg/L working standard.

Solution	*Volume of working STD (mL)*	*Volume of water (mL)*	*Conc. (µg/L)*
A	0	2000	0.0
B	5	1995	25
C	10	1990	50
D	20	1980	100
E	40	1960	200



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13. ABSTRACT (*Maximum 200 words*)

Methods for the detection and quantification of ammonium picrate and picric acid in soil and water were developed. Picrate ions were extracted from water directly or from acetone extracts of soil by solid-phase, acidic, ion-exchange materials. Elution from the ion exchangers was accomplished by converting the retained picrate to picric acid using strong aqueous, acid–organic solvent mixtures. The resulting colorless solution was then converted back to a colored picrate solution by dilution with water. Quantification and correction for background interferences were based on spectrophotometric measurements. A colorimetric, chemical confirmation of picrate was possible for the water method. The method detection limits were determined to be 1.3 µg/g for soil and 3.6 µg/L for water. Both methods can be implemented under field conditions.

14. SUBJECT TERMS

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